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(54) Title: VACCINATION AND METHODS AGAINST MULTIPLE SCLEROSIS RESULTING FROM PATHOGENIC RESPONSES BY SPECIFIC T CELL POPULATIONS		
(57) Abstract The present invention provides vaccines and a means of vaccinating a mammal so as to prevent or control specific T cell mediated pathologies or to treat the unregulated replication of T cells. The vaccine is composed of a T cell receptor (TCR) or a fragment thereof corresponding to a TCR present on the surface of T cells mediating the pathology. The vaccine fragment can be a peptide corresponding to sequences of TCRs characteristic of the T cells mediating said pathology. The vaccine is administered to the mammal in a manner that induces an immunologically effective response so as to affect the course of the disease. The invention additionally provides specific β -chain variable regions of the T cell receptor, designated V β 6.2/3, V β 6.5, V β 2, V β 5.1, V β 13 and V β 7, which are central to the pathogenesis of multiple sclerosis (MS).		

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VACCINATION AND METHODS AGAINST MULTIPLE SCLEROSIS
RESULTING FROM PATHOGENIC RESPONSES
BY SPECIFIC T CELL POPULATIONS

BACKGROUND OF THE INVENTION

5 This invention relates to the immune system and, more specifically, to methods of modifying pathological immune responses.

Higher organisms are characterized by an immune system which protects them against invasion by potentially
10 deleterious substances or microorganisms. When a substance, termed an antigen, enters the body, and is recognized as foreign, the immune system mounts both an antibody-mediated response and a cell-mediated response. Cells of the immune system termed B lymphocytes, or B
15 cells, produce antibodies which specifically recognize and bind to the foreign substance. Other lymphocytes termed T lymphocytes, or T cells, both effect and regulate the cell-mediated response resulting eventually in the elimination of the antigen.

20 A variety of T cells are involved in the cell-mediated response. Some induce particular B cell clones to proliferate and produce antibodies specific for the antigen. Others recognize and destroy cells presenting foreign antigens on their surfaces. Certain T cells
25 regulate the response by either stimulating or suppressing other cells.

While the normal immune system is closely regulated, aberrations in immune response are not uncommon. In some instances, the immune system functions inappropriately and
30 reacts to a component of the host as if it were, in fact, foreign. Such a response results in an autoimmune disease, in which the host's immune system attacks the host's own

tissue. T cells, as the primary regulators of the immune system, directly or indirectly effect such autoimmune pathologies.

Numerous diseases are believed to result from
5 autoimmune mechanisms. Prominent among these are
rheumatoid arthritis, systemic lupus erythematosus,
multiple sclerosis, Type I diabetes, myasthenia gravis and
pemphigus vulgaris. Autoimmune diseases affect millions of
individuals world-wide and the cost of these diseases, in
10 terms of actual treatment expenditures and lost
productivity, is measured in billions of dollars annually.
At present, there are no known effective treatments for
such autoimmune pathologies. Usually, only the symptoms
can be treated, while the disease continues to progress,
15 often resulting in severe debilitation or death.

In other instances, lymphocytes replicate
inappropriately and without control. Such replication
results in a cancerous condition known as a lymphoma.
Where the unregulated lymphocytes are of the T cell type,
20 the tumors are termed T cell lymphomas. As with other
malignancies, T cell lymphomas are difficult to treat
effectively.

Thus there exists a long-felt need for an effective
means of curing or ameliorating T cell mediated
25 pathologies. Such a treatment should ideally control the
inappropriate T cell response, rather than merely reducing
the symptoms. The present invention satisfies this need
and provides related advantages as well.

Summary of the Invention

30 The present invention provides vaccines and a means of
vaccinating a mammal so as to prevent or control specific
T cell mediated pathologies or to treat the unregulated

clonal replication of T cells. The vaccine is composed of a T cell receptor (TCR) or a fragment thereof corresponding to a TCR present on the surface of T cells mediating the pathology. The vaccine fragment can be a peptide
5 corresponding to sequences of TCRs characteristic of the T cells mediating said pathology. The vaccine is administered to the mammal in a manner that induces an immunologically effective response so as to affect the course of the disease.

10 The invention additionally provides specific β -chain variable regions of the T cell receptor, designated VB6.2/3, VB6.5, VB2, VB5.1, VB13 and VB7, which are central to the pathogenesis of multiple sclerosis (MS). Also provided are means to detect, prevent and treat MS.

15 Brief Description of the Figures

Figure 1 shows the frequency of the various TCR VB genes expressed in the cultures from CSF of MS patients.

Figure 2 shows the sequences of the VB gene family that are most frequently expressed in the cultures from CSF
20 of the MS patients.

Figure 3 shows a summary of the V β 7 CDR3 sequences. The top 3 monoclonal sequences were derived from cultured samples while the bottom sequences were obtained directly after sorting the CSF for CD25 (IL-2 receptor) and either
25 CD3 or CD4.

Detailed Description of the Invention

The invention relates to vaccines and their use for preventing or ameliorating T cell-mediated pathologies,

such as autoimmune diseases and T cell lymphomas. Vaccination provides a specific and sustained treatment which avoids problems associated with other potential avenues of therapy.

5 As used herein, the term "T cell-mediated pathology" refers to any condition in which an inappropriate T cell response is a component of the pathology. The term is intended to include both diseases directly mediated by T cells and those, such as myasthenia gravis, which are
10 characterized primarily by damage resulting from antibody binding, and also diseases in which an inappropriate T cell response contributes to the production of those antibodies. The term is intended to encompass both T cell mediated autoimmune diseases and unregulated clonal T cell
15 replication.

As used herein, "substantially the amino acid sequence," or "substantially the sequence" when referring to an amino acid sequence, means the described sequence or other sequences having any additions, deletions or
20 substitutions which do not substantially effect the ability of the sequence to elicit an immune response against the desired T cell receptor sequence. Such sequences commonly have many other sequences adjacent to the described sequence. A portion of the described immunizing sequence
25 can be used so long as it is sufficiently characteristic of the desired T cell receptor as to cause an effective immune response against desired T cell receptors but not against undesired T cell receptors. Such variations in the sequence can easily be made, e.g. by synthesizing an
30 alternative sequence, and tested, e.g. by immunizing a mammal, to determine its effectiveness.

As used herein, the term "fragment" is intended to cover such fragments in conjunction with or combined with additional sequences or moieties, as for example where the

peptide is coupled to other amino acid sequences or to a carrier. The terms "fragment" and "peptide" can, therefore, be used interchangeably since a peptide will be the most common fragment of the T cell receptor. Each
5 fragment of the invention can have an altered sequence, as described above for the term "substantially the sequence."

As used herein, the term "vaccine" means compositions which, when administered into an individual, affect the course of the disease by causing an effect on the T cells
10 mediating the pathology. This effect can include, for example, induction of cell mediated immunity or alteration of the response of the T cell to its antigen.

Reference herein to a "fragment or portion of the T cell receptor" does not mean that the composition must be
15 derived from intact T cell receptors. Such "fragments or portions" can be produced by various means well-known to those skilled in the art, such as for example manual or automatic peptide synthesis or methods of cloning.

As used herein when referring to the relationship
20 between peptide fragments of the invention and sequences of TCRs, "corresponding to" means that the peptide fragment has an amino acid sequence which is sufficiently homologous to the TCR sequence to stimulate an effective regulatory response in the individual. The sequence need not be
25 identical to the TCR sequence, however, as shown in Examples II and III.

By "substantially pure" it is meant that the TCR or fragment thereof is substantially free of other biochemical moieties with which it is normally associated in nature.
30 For example, the TCR is normally found with moieties derived from the same species of origin. Such moieties may act as undesirable contaminants when the TCR is used, for example, as a vaccine.

By "immunologically effective" is meant an amount of the T cell receptor or fragment thereof which, is effective to elicit a change in the immune response to prevent or treat a T cell mediated pathology or an unregulated T cell
5 clonal replication in the individual. Obviously, such amounts will vary between species and individuals depending on many factors. For example, higher doses will generally be required for an effective immune response in a human compared with a mouse.

10 As used herein, "binding partner" means a compound which is reactive with a TCR. Generally, this compound will be a Major Histocompatibility Antigen (MHC) but can be any compound so long as when the TCR is bound in the normal course, T cell activation or proliferation occurs.

15 As used herein, "ligand" means any molecule that reacts to form a complex with another molecule.

As used herein, "selectively binds" means that a molecule binds to one type of molecule but not substantially to other types of molecules. In relation to
20 VB17 "selective binding" indicates binding to VB17 containing TCRs but not substantially to other TCRs which lack VB17.

The immune system is the primary biological defense of the host (self) against potentially pernicious agents (non-
25 self). These pernicious agents may be pathogens, such as bacteria or viruses, as well as modified self cells, including virus-infected cells, tumor cells or other abnormal cells of the host. Collectively, these targets of the immune system are referred to as antigens. The
30 recognition of antigen by the immune system rapidly mobilizes immune mechanisms to destroy that antigen, thus preserving the sanctity of the host environment.

The principal manifestations of an antigen-specific immune response are humoral immunity (antibody mediated) and cellular immunity (cell mediated). Each of these immunological mechanisms are initiated through the activation of helper (CD4+) T Cells. These CD4+ T cells in turn stimulate B cells, primed for antibody synthesis by antigen binding, to proliferate and secrete antibody. This secreted antibody binds to the antigen and facilitates its destruction by other immune mechanisms. Similarly, CD4+ T cells provide stimulatory signals to cytotoxic (CD8+) T cells which recognize and destroy cellular targets (for example, virus infected cells of the host). Thus, the activation of CD4+ T cells is the proximal event in the stimulation of an immune response. Therefore, elaboration of the mechanisms underlying antigen specific activation of CD4+ T cells is crucial in any attempt to selectively modify immunological function.

T cells owe their antigen specificity to the T cell receptor (TCR) which is expressed on the cell surface. The TCR is a heterodimeric glycoprotein, composed of two polypeptide chains, each with a molecular weight of approximately 45 kD. Two forms of the TCR have been identified. One is composed of an alpha chain and a beta chain, while the second consists of a gamma chain and a delta chain. Each of these four TCR polypeptide chains is encoded by a distinct genetic locus containing multiple discontinuous gene segments. These include variable (V) region gene segments, junction (J) region gene segments and constant (C) region gene segments. Beta and delta chains contain an additional element termed the diversity (D) gene segment. (Since D segments and elements are found in only some of the TCR genetic loci, and polypeptides, further references herein to D segments and elements will be in parentheses to indicate the inclusion of these regions only in the appropriate TCR chains. Thus, V(D)J refers either

to VDJ sequences of chains which have a D region or refers to VJ sequences of chains lacking D regions.)

During lymphocyte maturation, single V, (D) and J gene segments are rearranged to form a functional gene that
5 determines the amino acid sequence of the TCR expressed by that cell. Since the pool of V, (D) and J genes which may be rearranged is multi-membered and since individual members of these pools may be rearranged in virtually any combination, the complete TCR repertoire is highly diverse
10 and capable of specifically recognizing and binding the vast array of binding partners to which an organism may be exposed. However, a particular T cell will have only one TCR molecule and that TCR molecule, to a large degree if not singly, determines the specificity of that T cell for
15 its binding partner.

Animal models have contributed significantly to our understanding of the immunological mechanisms of autoimmune disease. One such animal model, experimental allergic
20 encephalomyelitis (EAE), is an autoimmune disease of the central nervous system that can be induced in mice and rats by immunization with myelin basic protein (MBP). The disease is characterized clinically by paralysis and mild wasting and histologically by a perivascular mononuclear
25 cell infiltration of the central nervous system parenchyma. The disease pathogenesis is mediated by T cells with specificity for MBP. Multiple clones of MBP-specific T cells have been isolated from animals suffering from EAE and have been propagated in continuous culture. After in
30 vitro stimulation with MBP, these T cell clones rapidly induce EAE when adoptively transferred to healthy hosts. Importantly, these EAE-inducing T cells are specific, not only for the same antigen (MBP), but also usually for a single epitope on that antigen. These observations
35 indicate that discrete populations of autoaggressive T cells are responsible for the pathogenesis of EAE.

Analysis of the TCRs of EAE-inducing T cells has revealed restricted heterogeneity in the structure of these disease-associated receptors. In one analysis of 33 MBP-reactive T cells, only two alpha chain V region gene segments and a single alpha chain J region gene segment were utilized. Similar restriction of beta chain TCR gene usage was also observed in this T cell population. Only two beta chain V region segments and two J region gene segments were found. More importantly, approximately eighty percent of the T cell clones had identical amino acid sequences across the region of beta chain V-D-J joining. These findings confirm the notion of common TCR structure among T cells with similar antigen specificities and indicate that the TCR is an effective target for immunotherapeutic strategies aimed at eliminating the pathogenesis of EAE.

Various attempts have been made to exploit the antigen specificity of autoaggressive T cells in devising treatment strategies for EAE. For example, passive administration of monoclonal antibodies specific for TCRs present on EAE-inducing T cells has been employed. In the mouse model of EAE, infusion of a monoclonal antibody specific for V_β8, the major beta chain V region gene used by MBP-specific T cells, reduced the susceptibility of mice to subsequent EAE induction (Acha-Orbea et al., Cell 54:263-273 (1988) and Urban et al., Cell 54:577-592 (1988)). Similar protection has been demonstrated in rat EAE with monoclonal antibody reactive with an unidentified idiotypic determinant of the TCR on MBP specific T cells (Burns et al., J. Exp. Med. 169:27-39 (1989)). While passive antibody therapy appears to have some ameliorative effect on EAE susceptibility, it is fraught with potential problems. The protection afforded is transient, thus requiring repeated administration of the antibody. Multiple infusions of antibody increases the chances that the host will mount an immune response to the administered

antibody, particularly if it is raised in a xenogeneic animal. Further an antibody response to a pathogenic T cell clone represents only one element in the complete immune response and neglects the potential contributions of cellular immunity in resolving the autoreactivity.

The role of cellular immunity in reducing the activity of autoaggressive T cells in EAE has been examined and potential therapies suggested. In a manner similar to the passive antibody approach, regulatory T cells have been derived ex vivo and readministered for immunotherapy. For example, Sun et al., Nature, 332:843-845 (1988), have recently isolated a CD8+ T cell line from convalescing rats in whom EAE had been induced by adoptive transfer of an MBP-specific CD4+ T cell line. This CD8+ T cell line displayed cytolytic activity in vitro for the CD4+ T cell used to induce disease. Moreover, adoptive transfer of this CTL line reduced the susceptibility of recipient rats to subsequent challenge with MBP. Lider et al., Science, 239:181-183 (1988) have also isolated CD8+ T cells with suppressive activity for EAE-inducing T cells. These CD8+ cells were isolated from rats vaccinated with attenuated disease-inducing T cell clones and, though they showed no cytolytic activity in vitro, they could suppress MBP-driven proliferation of EAE-inducing T cells. Although these studies indicate that the CD8+ T cells could downregulate EAE, it is hard to reconcile a major role for these selected CD8+ CTLs in the long-term resistance of the recovered rats since Sedgwick, et al., (Eur. J. Immunol., 18:495-502 (1988)) have clearly shown that depletion of CD8+ cells with monoclonal antibodies does not affect the disease process or recovery.

In the experiments of Sun et al., and Lider et al., described above, the administration of extant derived regulatory T cells overcomes the major obstacle of passive antibody therapy; it permits a regulatory response in vivo

of prolonged duration. However, it requires in vitro cultivation with attenuated disease-inducing T cells to develop clones of such regulatory T cells, a costly and labor intensive process. Further, in an outbred population
5 such as humans, MHC non-identity among individuals makes this a highly individualized therapeutic strategy. Regulatory clones need to be derived for each individual patient and then re-administered only to that patient to avoid potential graft versus host reactions.

10 Direct vaccination with attenuated disease-inducing T cell clones also has been employed as a therapy for EAE. MBP-specific T cells, capable of transferring disease, have been attenuated by gamma irradiation or chemical fixation and used to vaccinate naive rats. In some cases,
15 vaccinated animals exhibited resistance to subsequent attempts at EAE induction (Lider et al., supra; see Cohen and Weiner, Immunol. Today 9:332-335 (1988) for review). The effectiveness of such vaccination, however, is inconsistent and the degree of protection is highly
20 variable. T cells contain a multitude of different antigens which induce an immune response when the whole T cell is administered as a vaccine. This phenomenon has been demonstrated by Offner et al., (J. Neuroimmunol., 21:13-22 (1989)), who showed that immunization with whole
25 T cells increased the delayed type hypersensitivity (DTH) response, as measured by ear swelling, to those T cells in an incremental manner as the number of vaccinations increased. However, positive DTH responses were found in both protected and non-protected animals. Rats responded
30 similarly to both the vaccinating encephalitogenic T cells and control T cells.

Conversely, vaccination with PPD-specific T cells from a PPD-specific T cell line induced DTH to the vaccinating cells as well as to an encephalitogenic clone even though
35 no protection was observed. The similar response of

vaccinated rats to both disease-inducing and control cells, as quantified by delayed-type hypersensitivity (a measure of cell-mediated immunity), indicates that numerous antigens on these T cells are inducing immune responses.

5 Thus, vaccination with attenuated disease-inducing T cells suffers from a lack of specificity for the protective antigen on the surface of that T cell, as well as, variable induction of immunity to that antigen. As a candidate for the treatment of human diseases, vaccination with

10 attenuated T cells is plagued by the same labor intensiveness and need for individualized therapies as noted above for infusion of CD8+ cells.

The present invention provides an effective method of immunotherapy for T cell mediated pathologies, including

15 autoimmune diseases such as multiple sclerosis, which avoids many of the problems associated with the previously suggested methods of treatment. By vaccinating, rather than passively administering heterologous antibodies, the host's own immune system is mobilized to suppress the

20 autoaggressive T cells. Thus, the suppression is persistent and may involve any and all immunological mechanisms in effecting that suppression. This multifaceted response is more effective than the uni-dimensional suppression achieved by passive administration of

25 monoclonal antibodies or extant-derived regulatory T cell clones.

As they relate to autoimmune disease, the vaccines of the present invention comprise TCRs of T cells that mediate autoimmune diseases. The vaccines can be whole TCRs

30 substantially purified from T cell clones, individual T cell receptor chains (for example, alpha, beta, etc.) or portions of such chains, either alone or in combination. The vaccine can be homogenous, for example, a single peptide, or can be composed of more than one type of

35 peptide, each of which corresponds to a different portion

of the TCR. Further, these peptides can be from distinct TCRs wherein both TCRs contribute to the T cell mediated pathology.

The VB6 TCR subunits were sequenced from 8 patients. From these 8 patients three-quarters (6 of 8) were identified as members of the VB5.2/3 and VB6.5 subfamily shown in Figure 2. These two subfamilies of the VB6 gene family show considerable homology in the CDR2 region between residues 39 and 58. It appears that these two particular members of the VB6 family are particularly associated with multiple sclerosis.

In a further specific embodiment, T cell receptors, whole T cells or fragments of the TCR which contain the VB chains designated VB6.2/3, VB6.5, VB2, VB5.1, VB13, VB7 can be used to immunize an individual having or at risk of having multiple sclerosis to treat or prevent the disease. The immune response generated in the individual can neutralize or kill T cells having the particular VB subunit and, thus, prevent or treat the deleterious effects of the VB-bearing T cells. Moreover, to the extent that these VB subunits are common to T cell receptors on pathogenic T cells mediating autoimmune diseases in general, such vaccines can also be effective in ameliorating such other autoimmune diseases.

The vaccines comprise peptides of varying lengths corresponding to the TCR or portions thereof. The peptides can be produced synthetically or recombinantly, by means well known to those skilled in the art. Preferably, the peptide vaccines correspond to regions of the TCR which distinguish that TCR from other nonpathogenic TCRs. Such specific regions can be located within the various region(s) of the respective TCR polypeptide chains, or spanning the various regions such as a short sequence spanning the V(D)J junction, thus restricting the immune

response solely to those T cells bearing this single determinant.

The vaccines are administered to a host exhibiting or at risk of exhibiting an autoimmune response. Definite
5 clinical diagnosis of a particular autoimmune disease warrants the administration of the relevant disease-specific TCR vaccines. Prophylactic applications are warranted in diseases where the autoimmune mechanisms precede the onset of overt clinical disease. Thus,
10 individuals with familial history of disease and predicted to be at risk by reliable prognostic indicators could be treated prophylactically to interdict autoimmune mechanisms prior to their onset.

TCR vaccines can be administered in many possible
15 formulations, in pharmacologically acceptable mediums. In the case of a short peptide, the peptide can be conjugated to a carrier, such as KLH, in order to increase its immunogenicity. The vaccine can be administered in conjunction with an adjuvant, various of which are known to
20 those skilled in the art. After initial immunization with the vaccine, a booster can be provided. The vaccines are administered by conventional methods, in dosages which are sufficient to elicit an immunological response, which can be easily determined by those skilled in the art.

25 Appropriate peptides to be used for immunization can be determined as follows. Disease-inducing T cell clones reactive with the target antigens are isolated from affected individuals. Such T cells are obtained preferably from the site of active autoaggressive activity such as a
30 lesion in the case of pemphigus vulgaris, central nervous system (CNS) in the case of multiple sclerosis or synovial fluid or tissue in the case of rheumatoid arthritis, or alternatively from blood of affected individuals. The TCR genes from these autoaggressive T cells are then sequenced.

Polypeptides corresponding to TCRs or portions thereof that are selectively represented among disease inducing T cells (relative to non-pathogenic T cells) can then be selected as vaccines and made and used as described above.

- 5 Alternatively, the vaccines can comprise anti-idiotypic antibodies which are internal images of the peptides described above. Methods of making, selecting and administering such anti-idiotypic vaccines are well known in the art. See, for example, Eichmann, et al., CRC Critical
10 Reviews in Immunology 7:193-227 (1987), which is incorporated herein by reference.

Multiple Sclerosis

- T cells causative of multiple sclerosis (MS) have not previously been identified, though MBP-reactive T cells
15 have been proposed to play a role due to the clinical and histologic similarities between MS and EAE. In rat and mouse models of EAE, MBP-reactive, encephalogenic T cells show striking conservation of β -chain VDJ amino acid sequence, despite known differences in MHC restriction and
20 MBP-peptide antigen specificity. This invention is premised on the observation that a human myelin basic protein (MBP)-reactive T cell line, derived from an MS patient, has a TCR β -chain with a VDJ amino acid sequence homologous with that of β -chains from MBP-reactive T cells
25 mediating pathogenesis in experimental allergic encephalomyelitis (EAE), an animal model of MS. This line is specific for another epitope of MBP. This finding demonstrates the involvement of MBP-reactive T cells in the pathogenesis of MS and demonstrates that TCR peptides
30 similar to those described herein for the prevention of EAE can be appropriate in treating MS.

Specifically, the invention provides a method of diagnosing or predicting susceptibility to T cell mediated

pathologies in an individual comprising detecting T cells having the β -chain variable regions designated VB6.2/3, VB6.5, VB5.1, VB7, VB13, or VB2 in a sample from the individual, the presence of abnormal levels of these VB-containing T cells indicating the pathology or susceptibility to the pathology. The VB containing T cell can be qualitatively or quantitatively compared to that of normal individuals. Such diagnosis can be performed for example by detecting a portion of the VB17 which does not occur on multiple sclerosis associated β -chain variable region T-cell receptors. The VB17 can be detected, for example, by contacting the VB17 with a detectable ligand capable of specifically binding to VB17. Many such detectable ligands are known in the art, e.g. an enzyme linked antibody. Alternatively, nucleotide probes complementary to the VB subunit-encoding nucleic acid sequences can be utilized to detect T cells containing the corresponding VB subunit, as taught in Examples VIII and IX.

20 The invention also provides a method of preventing or treating a T cell mediated pathology comprising preventing the attachment of the VB subunit containing T-cell receptor to its binding partner. In one embodiment attachment is prevented by binding a ligand to the VB subunit. In an alternative embodiment attachment is prevented by binding a ligand to the binding partner. Attachment can be prevented by known methods, e.g. binding an antibody to the subunit or the binding partner to physically block attachment.

30 The invention also provides a method of preventing or treating a T cell mediated pathology in an individual comprising cytotoxicly or cytostatically treating T-cells containing the particular VB subunit in the individual. In one embodiment, the VB containing T-cells are treated with a cytotoxic or cytostatic agent which selectively binds

VB17. The agent can be an antibody attached to a radioactive or chemotherapeutic moiety. Such attachment and effective agents are well known in the art. See, for example, Harlow, E. and Lane, Antibodies, A Laboratory
5 Manual, Cold Spring Harbor Laboratory, 1988, which is incorporated herein by reference.

The invention also provides a method of preventing or treating multiple sclerosis in an individual comprising cytotoxicly or cytostatically treating T cells containing
10 substantially the SGDQGGNE sequence in the individual. In one embodiment, T-cells are treated with a cytotoxic or cytostatic agent which selectively binds the sequence. The agent can be an antibody attached to a radioactive or chemotherapeutic moiety.

15 The following examples are intended to illustrate but not limit the invention.

EXAMPLE I

RAT MODEL OF EAE

Female Lewis rats, (Charles River Laboratories,
20 Raleigh-Durham, NC) were immunized in each hind foot pad with 50 μ g of guinea pig myelin basic protein emulsified in complete Freund's adjuvant. The first signs of disease were typically observed 9-11 days post-immunization. Disease severity is scored on a three point scale as
25 follows: 1=limp tail; 2=hind leg weakness; 3=hind leg paralysis. Following a disease course of approximately four to six days, most rats spontaneously recovered and were refractory to subsequent EAE induction.

EXAMPLE II
SELECTION AND PREPARATION OF VACCINES

Vaccinations were conducted with a T cell receptor peptide whose sequence was deduced from the DNA sequence of a T cell receptor beta gene predominating among EAE-inducing T cells of B10.PL mice. The DNA sequence was that reported by Urban, et al., supra, which is incorporated herein by reference. A nine amino acid peptide, having the sequence of the VDJ junction of the TCR beta chain of the mouse, was synthesized by methods known to those skilled in the art. The sequence of this peptide is: SGDAGGGYE. (Amino acids are represented by the conventional single letter codes.) The equivalent sequence in the rat has been reported to be: SSD-SSNTE (Burns et al., J. Exp. Med. 169:27-39 (1989)). The peptide was desalted by Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ) column chromatography in 0.1 M acetic acid and the solvent was subsequently removed by two cycles of lyophilization. A portion of the peptide was conjugated to keyhole limpet hemocyanin (KLH) with glutaraldehyde at a ratio of 7.5 mgs of peptide per mg of KLH. The resulting conjugate was dialyzed against phosphate buffered saline (PBS).

EXAMPLE III
VACCINATION AGAINST EAE

Vaccines used in these studies consisted of free VDJ peptide and also of VDJ peptide conjugated to KLH. These were dissolved in PBS and were emulsified with equal volumes of either (1) incomplete Freund's adjuvant (IFA) or (2) complete Freund's adjuvant (CFA) made by suspending 10 mg/ml heat killed desiccated Mycobacterium tuberculosis H37ra (Difco Laboratories, Detroit, MI) in IFA. Emulsions were administered to 8-12 week old female Lewis rats in a

final volume of 100 microliters per animal (50 μ l in each of the hind footpads). 5 μ g of unconjugated VDJ peptide were administered per rat. KLH-VDJ conjugate was administered at a dose equivalent to 10 μ g of KLH per rat.

5 Twenty-nine days later each rat was challenged with 50 μ g
of guinea pig myelin basic protein in complete Freund's
adjuvant in the front footpads. Animals were monitored
daily beginning at day 9 for clinical signs of EAE and were
scored as described above. The results are presented in
10 Table I. As can be seen, not only was there a reduced
incidence of the disease in the vaccinated individuals, but
in those which did contract the disease, the severity of
the disease was reduced and/or the onset was delayed. The
extent of protection varied with the vaccine formulation,
15 those including CFA as the adjuvant demonstrating the
greatest degree of protection.

TABLE I

[illegible]

EXAMPLE IVVaccination against EAE with Lewis Rat VDJ peptides

The VDJ peptide used in the previous examples was synthesized according to the sequence of TCR B chain molecules found on EAE-inducing T cells in B10.PL mice. In addition, peptides were synthesized and tested which correspond to sequences found on encephalitogenic T cells in Lewis rats. These VDJ sequences are homologous with that of B10.PL mice, but not identical. The rat peptides were synthesized according to the DNA sequences reported by Burns, et al. and Chluba, et al., Eur. J. Immunol. 19:279-284 (1989). The sequences of these peptides designated IR1, 2, 3 and 9b are shown below, aligned with the B10.PL mouse sequence used in Examples I through III (VDJ).

15	VDJ	S G D A G G Y E
	IR1	C A S S D - S S N T E V F F G K
	IR2	C A S S D - S G N T E V F F G K
	IR3	C A S S D - S G N - V L Y F G E G S R
	IR9b	A S S D - S S N T E

20 The preparation, administration and evaluation of these vaccines were conducted as described in Examples I through III with the following exceptions: 50 μ g of the individual VDJ peptides were incorporated into vaccine formulations containing CFA; neither vaccinations in IFA nor vaccinations with peptides conjugated to KLH were conducted. Control animals were untreated prior to MBP challenge as in Example III or were vaccinated with emulsions of PBS and CFA to assess the protective effect of adjuvant alone. The results are shown in Table II below.

TABLE II

	Animal No.	Vaccination (Adjuvant)	Days After Challenge									
			10	11	12	13	14	15	16	17	18	
5	1	None	-	1	2	3	3	2	-	-	-	
	2	"	1	3	3	3	2	-	-	-	-	
	3	"	-	2	3	3	3	1	-	-	-	
10	4	PBS-CFA	1	2	3	3	3	-	-	-	-	
	5	"	1	2	3	3	3	-	-	-	-	
	6	"	-	2	3	3	3	-	-	-	-	
	7	IR1 (50 µg)	-	-	-	2	1	-	-	-	-	
	8	"	-	-	-	-	1	3	-	-	-	
	9	"	-	-	-	1	1	1	1	-	-	
15	10	IR2 (50µg)	-	-	1	3	3	3	-	-	-	
	11	"	-	-	-	-	2	2	3	3	-	
	12	"	-	-	-	-	1	-	-	-	-	
	13	IR3 (50µg)	1	3	3	3	2	-	-	-	-	
	14	"	-	-	2	3	3	-	-	-	-	
	15	"	-	-	-	-	-	-	-	-	-	
20	16	IR9b (50 µg)	-	-	-	-	-	-	-	-	-	
	17	"	-	-	-	-	-	-	-	-	-	
	18	"	-	-	-	-	-	-	-	-	-	
	19	"	-	-	-	-	-	-	-	-	-	
25	Scoring:	-	no signs									
		1)	limp tail									
		2)	hind leg weakness									
		3)	hind leg paralysis									

As shown in Table II, disease in unvaccinated control animals was observed as early as day 10. Disease was characterized by severe paralysis and wasting, persisted for 4 to 6 days and spontaneously remitted. PBS-CFA vaccinated rats displayed disease courses virtually indistinguishable from those of unvaccinated controls. In contrast, delays in onset were observed in some of the IR1, 2 or 3 vaccinated animals and others showed both delayed onset as well as decreased severity and/or duration of disease. Overall, however, vaccinations with the rat VDJ peptides (IR1-3) were slightly less effective than those

with the mouse VDJ peptide (Example III). Vaccination with IR9b, however, afforded complete protection in all four animals in which it was tested. Importantly, no histologic lesions characteristic of disease were found in any of the
5 four animals vaccinated with IR9b indicating that sub-clinical signs of disease were also abrogated.

EXAMPLE V

Vaccination with V region specific peptides

A peptide specific for the VB8 gene family was tested
10 as a vaccine against EAE. VB8 is the most common β chain gene family used by encephalitogenic T cells in both rats and mice. A peptide was synthesized based on a unique DNA sequence found in the VB8 gene, and which is not found among other rat VB genes whose sequences were reported by
15 Morris, et al., Immunogenetics 27:174-179 (1988). The sequence of this VB8 peptide, designated IR7, is:

IR7 D M G H G L R L I H Y S Y D V N S T E K

The efficacy of this VB8 peptide was tested in the Lewis rat model of EAE (Example I) as described in Examples
20 II and III. 50 μ g of peptide were tested in CFA. Vaccinations in IFA or with peptide-KLH conjugates were not conducted. The results of these studies are shown in Table III.

TABLE III

Animal No.	Vaccination (Adjuvant)	Days After Challenge									
		10	11	12	13	14	15	16	17	18	
5	1	IR7 (50 µg)	-	-	1	2	3	3	3	-	-
	2	"	-	-	-	-	1	1	-	-	-
	3	"	-	-	-	-	-	-	-	-	-
10	Scoring:	-	no signs								
		1)	limp tail								
		2)	hind leg weakness								
		3)	hind leg paralysis								

The results of vaccinations conducted with the rat VB8 peptide are similar to those observed with the mouse and rat IR1, 2 and 3 peptides. Delayed onset as well as decreased severity and duration of disease was observed in one animal. One animal was completely protected.

EXAMPLE VIVaccination with J region peptides

A peptide was synthesized which corresponds to the J α gene segment, TA39, found among both rat and mouse encephalitogenic T cell receptors. The sequence of this peptide, designated IR5, is:

IR5 R F G A G T R L T V K

The efficacy of the J α TA39 peptide was tested in the Lewis rat model of EAE (Example I) as described in Examples II and III. 50 µg of peptide were tested in CFA. Vaccinations in IFA or with peptide-KLH conjugates were not conducted. The results of these studies are shown in Table IV.

TABLE IV

Animal No.	Vaccination (Adjuvant)	Days After Challenge										
		10	11	12	13	14	15	16	17	18	19	20
5	1	IR5 (50 µg)										
	2	"										
	3	"										
Scoring:		-	no signs									
10		1)	limp tail									
		2)	hind leg weakness									
		3)	hind leg paralysis									

The results of vaccinations conducted with the rat J α TA39 peptide are more effective than those observed with the mouse VDJ peptide or the V β 8 peptide. Two of three animals were totally protected and, in the third, disease onset was markedly delayed. Severity was also reduced in this animal though disease persisted for a normal course of 5 days. Importantly, the two animals which were completely protected showed no histologic evidence of T cell infiltration of the CNS. This result indicates that vaccinating with the J α TA39 very efficiently induces a regulatory response directed at encephalitogenic T cells. Even sub-clinical signs of disease were abrogated.

25

EXAMPLE VIIVaccination with mixtures of TCR peptides

Vaccinations were conducted with a mixture of TCR peptides. This mixture contained 50 µg of each of the peptides IR1, 2, 3 and 5 (the three rat VDJ peptides and the rat J α TA39 peptide).

30

The efficacy of this peptide mixture was tested in the Lewis rat model (Example I) as described in Examples II and III. Peptides were tested in CFA. Vaccinations in IFA or

25

with peptide-KLH conjugates were not conducted. The results of these studies are shown in Table V.

TABLE V

5	Animal No.	Vaccination (Adjuvant)	Days After Challenge									
			10	11	12	13	14	15	16	17	18	
	4	IR1, 2, 3, 5	-	-	-	-	-	-	-	-	-	
	5	(50 µg each)	-	-	-	-	-	-	-	-	-	
	6	"	-	-	-	-	-	-	-	-	-	
10												
	Scoring:	-	no signs									
		1)	limp tail									
		2)	hind leg weakness									
		3)	hind leg paralysis									

15 The results of vaccinations conducted with the rat J α TA39 and three VDJ peptides were as effective as those described for IR9b in Table II. All three animals were totally protected. In addition to the absence of any clinical signs of EAE, two of these three animals were

20 completely free of histological evidence of T cell infiltration into the CNS while the third showed only two small foci of lymphocytic infiltration at the base of the spinal cord.

EXAMPLE VIII

25

Multiple Sclerosis Vaccine

CSF Cells: Cerebrospinal fluid (CSF) was obtained from 28 patients who were tapped at least once. Twelve were tapped twice and one tapped three times. In addition three

30 patient's CSF cells were cultured and assessed in duplicate. Fifty-150 thousand lymphocytes were recovered from 20 ml CSF; these cells were spun down and resuspended in 200 µl human T cell media (HTC) which consists of RPMI 1640 supplemented with human AB serum (15%), glutamine (2mM), HEPES buffer (10mM), 2-mercaptoethanol (0.05mM), and

the antibiotics penicillin and streptomycin (each 5iu/ml). A small aliquot (20 thousand cells) was set aside for flow cytometric analysis following staining with monoclonal antibodies Mabs specific for CD25 (IL-1R), CD4 and CD8, and
5 irrelevant (control antibodies) directly coupled to fluorescein isothiocyanate (FITC) and phycoerythrin (PE).

The remaining cells (30-130 thousand) were exposed to washed DYNABEADS (Dynal Inc., Great Neck, N.Y.) (ratio 1:20) coupled with Mab specific for human CD8 α chains. CD8⁺
10 T cells coupled to these beads stick to the walls of the tube in a magnetic field and CD8⁻ T cells were recovered by pipetting off the fluid. Generally, this provides a yield of 70-80% (20-100 thousand cells) with greater than 95% depletion of cells bearing the CD8 marker.

15 The remaining cell population consists, at this point mostly of CD4⁺ T cells, half or more of which are activated and express the CD25 marker (IL-2R). These activated T cells were expanded in cultures (20-50 thousand cells per well) of HTC medium supplemented with 20% Lymphocult-T-LF
20 (Biotest Diagnostics Corp.; Denville, N.J.) and with recombinant human IL-2 and IL-4 (R & D Systems, Minneapolis, MN) (50 μ /ml). Cultures were fed twice weekly; generally, after 1 week, cells began to overgrow their cultures. Each well was split into three, and four
25 days later into six, wells. After 10 days to 2 weeks of culture, the initial inoculum generated more than 1 million cells. Flow cytometric analysis of the cultured cells indicated more than 95% were CD4⁺CD8⁻CD3⁺TCR α β ⁺. Occasionally a majority of cells were CD4⁻CD8⁻CD3⁺, a
30 population assumed to be rich in TCR γ δ ⁺ T cells.

Total cellular RNA was isolated from T cell populations following lysis in guanidinium isothiocyanate and phenol extraction (Chomczynski, P., and N. Sacchi, "Single-Step Method of RNA Isolation by Acid Guanidinium

Thiocyanate-Phenol-Chloroform Extraction," Analy. Biochem. 162:156 (1987), which is incorporated herein by reference. The RNA (3-5 μ g) was first denatured in methyl mercuric hydroxide (10mM final concentration; Alfa Products, Ward Hill, MA) and then converted to cDNA in Taq (*Thermus aquaticus*) DNA polymerase buffer (Perkins Elmer Cetus; Norwalk, CT) (50mM KCl, 10mM Tris-HCl pH8.3, 2.5mM MgCl₂, and 0.01% gelatin) in the presence of RNasin (20 units, Promega, Madison, WI), β -mercaptoethanol (40mM), dNTPs (0.5mM, Pharmacia), C β specific oligonucleotide primer (C β -1; 1 μ M) and AMV (Avian myeloblastosis virus) reverse transcriptase (8 units, U.S. Biochemical, Cleveland, OH) in a 25 μ l reaction for 90 minutes at 42°C. The C β oligonucleotide primer, (C β -1), is complementary to a sequence found at the C terminal of human C β 1 and C β 2 mRNA.

PCR amplification: cDNA was transferred to a tube containing the following: a C β -2 primer (0.6 μ M) corresponding to a sequence more internal to C β -1 used in cDNA synthesis, dNTPs (200 μ M) and Taq DNA polymerase (23u, Perkin Elmer Cetus, Norwalk, CT), in Taq polymerase buffer as above except for the presence of 1.5mM MgCl₂. Fifty μ l of this mixture is added to each of 30 individual wells of a microtest III U bottom flexible assay plate (Falcon, 3911; Becton Dickinson and Co., Oxnard, CA). Each well contained a different oligonucleotide V β primer specific for one of the 30 known human V β families shown in the accompanying table (0.6 μ M in 1 μ l) or no V β primer as control. The wells were overlain with light mineral oil (Sigma; St. Louis, MO), heated to 94°C for 5 minutes to denature DNA/RNA duplexes and then subjected to 27 amplification cycles of 1 minute at 94°C for melting, 1.5 minutes at 55°C for annealing, and 2 minutes at 72°C for extension in a 96 well thermal cycler (MJ Research, Inc.; Watertown, MA).

Quantitation of V β expression: Following amplification, 15 μ l of the PCR product was denatured for

20 minutes at room temperature by the addition of 15 μ l 1N NaOH. The samples were then neutralized by the addition of 15 μ l 1N HCl and 15 μ l 20X SSC. 15 μ l of the neutralized samples was spotted onto nitrocellulose filters (BA85, Schleicher & Schuell; Keene, NH) using a Bio-Dot microfiltration apparatus (Bio-Rad Laboratories; Richmond, CA) and then cross-linked to the filter using a UV Stratalinker 1800 according to manufacturer's recommendations (Stratagene; San Diego, CA). The relative level of amplification in each well was assessed by probing with a gamma 32 P (DuPont; Boston, MA) end-labelled C β specific oligonucleotide, C β -3, which was further 5' to the C β oligonucleotide used in the PCR. Filters were pre-hybridized at 37°C for 1-2 hours in a mixture containing 6X SSC, 1X Denhardt's solution, 0.5% SDS, 0.05% sodium pyrophosphate, and 100 μ g/ml sonicated salmon sperm DNA (Salmon sperm DNA Cat.#1626; Sigma; St. Louis, MO). The filters were then hybridized with the radiolabeled oligonucleotide C β primer overnight at 37°C in a mixture containing 6X SSC, 1X Denhardt's, 0.1% SDS, 0.05% sodium pyrophosphate and 20 μ g/ml wheat germ tRNA (Type V, Sigma; St. Louis, MO). Following hybridization, the filters were washed twice at 37°C for 30 minutes in 6X SSC containing 0.05% sodium pyrophosphate and one time more at 47°C for 10 minutes. The level of hybridization for each V β was measured using an AMBIS radioisotope detector (Ambis; San Diego, CA). All values are corrected by subtracting counts incorporated into the water blank control well. Relative V β expression was calculated by summing all counts detected and dividing this value into the net counts for any given well.

A summary of results of TCR β chain usage among T cells in the CSF of MS patients is presented in Figure 1. As can be seen, VB2 was expressed on greater than 70% of the cultured CSF T cells from 1 patient, 50-59% of T cells from another, 20-29% from 4 others, and so forth.

Assuming 30-50 different VB members in the repertoire of human T cell receptors, each being represented randomly, the frequency of any one TCR VB member would be expected to be approximately 2-3% of the total in a given T cell population. In fact, one rarely finds expression of any particular VB chain exceeding 10% in the peripheral blood T cell pool of a normal individual. Figure 1 indicates the disproportionate usage (greater than 20%) of at least 4 different VB members among the T cells of CSF from MS patients.

In order of most frequent usage (greater than 20%) VB6.2/3 or VB6.5 are used disproportionately in 13 samples, VB2 in 6 samples, VB5.1 and VB13 in 4 samples each. Thus, among 39 samples, 27 of them show a disproportionate usage of one or more of these 4 different TCR VB members.

The predicted amino acid sequences of these VB members is shown in Figure 2.

Based on preliminary data gathered over the past year, a peptide of the CDR2 region of VB6.5 encompassing amino acids 39 - 58 was made 39 - LeuGlyGlnGlyProGluPheLeuThrTyrPheGlnAsnGluAlaGlnLeuGluLys Ser-58. (LGQGPEFLTYFQNEAQLEKS) This region is nearly identical to the corresponding sequences found in VB6.2/3, VB6.8 and VB6.9, and differs only slightly from sequences found in VB6.4, VB6.7, and VB6w1. This peptide is effective in provoking an immune response against TCR of most of the VB6 family.

Of the 13 samples (from 12 patients) where VB6.2/3 or VB6.5 was used heavily, sequence studies have been conducted on material from 8 patients to determine (i) the degree of clonality based on homogeneity of sequences in

the CDR3 junctional region and (ii) which of the many members of the VB6.2/3 and VB6.5 family is involved.

DNA Sequencing. To determine the DNA sequence of the expressed VB, the PCR reaction was repeated as described
5 above for 30 cycles with CB-1 and the VB6 specific primer. Following amplification, the resulting PCR products were first made blunt-ended by addition of 5 units T4 DNA polymerase (Pharmacia Fine Chemicals; Piscataway, N.J.) for 15 minutes at 37°C, then extracted with chloroform to
10 remove mineral oil, precipitated with ethanol, and digested with EcoRI according to manufacturer's specifications (New England Biolabs; Beverly, MA); the resulting DNA was separated on a 1.4% agarose gel (Ultra PURE Agarose, GIBCO BRL; Gaithersburg, MD). The appropriate size product was
15 Isolated using Prep-A-Gene (Bio-Rad; Richmond, CA), ligated into the HincII/EcoRI site of pBluescript II (Stratagene; San Diego, CA), and the ligation mixture was then transformed into the bacterial strain DH5 (available from GIBCO, BRL). Multiple ampicillin resistant colonies were
20 selected and miniprep DNA was prepared by standard methods (Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, New York which is incorporated herein by reference). The plasmid DNA was then sequenced directly by
25 dideoxy chain termination (Sanger, F., S. Nicklen, and A.R. Coulson, "DNA sequencing with chain-terminating inhibitors." Proc. Natl. Acad. Sci. USA. 78:5453 (1977), which is incorporated herein by reference, using the Sequenase sequencing method (U.S. Biochemical, Cleveland,
30 OH).

These studies established that VB utilization is indeed clonal, displaying shared CDR3 sequences on a majority of the PCR amplified material, and that a single VB6.2/3 and VB6.5 family member dominates. To date, of the
35 8 patients, 4 use VB6.5, 1 uses VB6.4, 2 use VB6.2, and 1

uses V β 6.1. These results indicate that the peptide vaccine would be effective for 6 or 7 of the 8 patients.

EXAMPLE IX

A study was performed on CSF samples from MS patients
5 in which V β 7 was a dominant clone in 3 out of a total of 5 samples. The protocol used was parallel to that provided in Example VIII with the following modifications. The oligonucleotide primers have different amplification efficiencies and can amplify additional TCR V β subfamily
10 members than those described above. In addition, the CSF samples were not depleted of CD8+ cells.

Unfractionated CSF were cultured in single or duplicate microtiter wells for 14-23 days in IL-2/IL-4 and Lymphocult (Biotest Diagnostics Corp.; Denville, N.J.)
15 (hereafter referred to as the "culture") as described above. The T cell subset phenotyping was performed after this culture period and is summarized in Table XI as the percentage each of CD4+ and CD8+ T cells. For patients 88 and 94, the CSF samples were split into 2 wells prior to
20 culturing which is noted as well 1 and well 2. It is clear from this that in nearly all CSF and PBL samples, over 50% of the cultured cells were CD4+, which is desirable since it is this population which is of most interest.

In patient 82 a TCR usage was dominated by V β 7 and 18,
25 which account for approximately 65% of the total signal (as assessed by Ambis scanning). This dominant or "restricted" V β usage was explored further by sequencing the CDR3 domain, the most variable region of the TCR β chain and one believed to be important in binding antigen. Table XIII
30 shows that the dominant V β 7 of the PCR arose from one cell as assessed by the monoclonal (11/11 clones sequenced were identical) CDR3 domain. V β 18 was oligoclonal in that 2

cells contributed to the PCR product as seen by 2 discrete CDR3 domains.

Surprisingly, when the material from patient 88 was equally split into 2 wells and treated identically, 5 different dominant clones arose. Well 1 was dominated by V β 14 while well 2 had dominant V β 7 and 20. Table 4 reveals the CDR3 domains of several of the clones growing in each well and indicates that each was clonal. Restricted V β usage in the cultured CSF samples was not true for the 10 patient's PBLs cultured at similar densities (Figure 3). The V β profile for the PBLs were more diverse, where more of the signal was contributed by a wider variety of T cells.

The CSF sample from patient 94 was again split into 2 15 replicate samples prior to culture. Like the previous samples, there is evidence of a restricted V β usage by clones expanded by the culture conditions. Both populations were over 75% CD4+ (Table 2). However, like CSF 88, there were different dominant clones growing in 20 each well although V β 3 was dominant in both wells. Table 5 reveals that while the CDR3 domains of V β 3 for each clone are monoclonal, they are discrete rearrangements, indicating that they did not descend from one progenitor. This observation is presently being investigated. 25 Figure 5 shows the cultured PBLs from patient 94 which demonstrates a broad array of TCR V β usage. For these samples, different seeding cell densities (40,000 versus 200,000) were found to yield different V β profiles.

The cultured CSF and PBLs from patient 95 contained a 30 dominant V β 7, which accounted for approximately 65% of the total signal. The normal range in blood for V β 7 is 10-11%. There is clearly a preferential expansion of V β 7+ CSF cells under the present culture conditions. Although several other V β bands can be seen, they account for a relatively

small percentage of the total. The PBLs from this patient, like the others, is quite diverse, with no dominance noted. Table 7 shows the CDR3 domain of V β 7 from patient 95 and demonstrates that a single cell arose during culturing.

5

The last sample, 101 was actually sorted for CD4 and HLA DR positivity after culture but prior to PCR analysis. The CSF clearly contained 4-5 dominant V β s. Interestingly, this was the first PBL sample to show a restricted profile
: 10 which may be due to sorting the sample after culture.

Although a limited number of MS patients were examined it appears that V β 7 is overrepresented in the CSF T cell population cultured with IL-2, IL-4, and Lymphocult.

Although the invention has been described with regard
15 to present embodiments, the invention is not limited except by the claims.

WE CLAIM:

1. A vaccine for preventing or treating multiple sclerosis in a mammal, comprising an immunologically effective amount of a T cell receptor, or a fragment thereof, corresponding to the amino acid sequence of VB6.2/3, VB6.5 a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.
2. A vaccine for preventing or treating multiple sclerosis a mammal, comprising an immunologically effective amount of a T cell receptor, or a fragment thereof, corresponding to the amino acid sequence of VB2 a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.
3. A vaccine for preventing or treating multiple sclerosis a mammal, comprising an immunologically effective amount of a T cell receptor, or a fragment thereof, corresponding to the amino acid sequence of VB5.1 a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.
4. A vaccine for preventing or treating multiple sclerosis a mammal, comprising an immunologically effective amount of a T cell receptor, or a fragment thereof, corresponding to the amino acid sequence of VB13 a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.
5. A vaccine for preventing or treating multiple sclerosis a mammal, comprising an immunologically effective amount of a T cell receptor, or a fragment thereof, corresponding to the amino acid sequence of VB7 a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.

6. The vaccine of claim 1-5, further comprising an adjuvant.

7. The vaccine of claim 1-5, wherein said vaccine comprises more than one type of T cell receptor or fragment thereof.

8. The vaccine of claim 1-5, wherein said vaccine comprises more than one fragment corresponding to different sequences of the same T cell receptor.

9. The vaccine of claim 1-5, wherein said fragment is conjugated to a carrier.

10. A method of vaccinating an individual exhibiting or at risk of exhibiting a T cell-mediated pathology, comprising administering to the individual the vaccine of claim 1-5.

11. The method of claim 19, wherein said vaccine is administered more than once.

12. The method of claim 10, wherein said vaccine is administered in a formulation including an adjuvant.

13. A method of diagnosing or predicting susceptibility to multiple sclerosis in an individual comprising detecting T cells having the β -chain variable region designated VB17 or a fragment thereof in a sample from the individual, the presence of abnormal expression of VB6.2/3-, VB6.5-, VB2-, VB5.1-, VB13-, VB7-containing T cells indicating said pathology or susceptibility to said pathology.

14. The method of claim 13, wherein the presence of said VB is detected by a nucleotide probe which is complementary to the nucleotide sequence of the VB subunit.

15. A method of preventing or treating multiple sclerosis comprising preventing the attachment of a VB6.2/3, VB6.5, VB2, VB5.1, VB13, VB7 containing T-cell receptor to its binding partner.
- 5 16. A method of preventing or treating multiple sclerosis in an individual comprising cytotoxically or cytostatically treating VB17 containing T-cells in the individual.

1/5

	V beta														
%	1	2	3	4	5.1	5.2	6	7.2	8.1	8.3	9	10	11	12.1	
70		1					2								
60-69															
50-59	1						1								
40-49				2			2								
30-39				2			2	1							
20-29	4	1	1	1		1	6			2					
10-19	1	5	4	2	4	2	11	1	5		2			6	
5-9	2	16	11	4	7	1	9	7	5	2	5	1	1	10	
0-4	36	12	23	32	24	35	6	30	29	37	30	38	38	23	

FIG.1A

2/5

v beta		12.2	13	13.5	14	15	16	17	18	19	20	21.1	21.2	21.3	22	23	24
70																	
60-69		1															
50-59																	
40-49		1										1					
30-39	3	2			1	1											
20-29					1	1						2					
10-19		10		3					2		1			1			1
5-9	1	9		7	3				6	1	1		5	4	2	3	
0-4	37	16	39	39	29	34	39	37	31	38	37	39	30	35	36	35	38

FIG.1B

	1	10	20	30	40	50	60	70	80	90
HUVB6.1	MGTRLLCWAALCLLGADHTGAGVSTPSNVTEKGKYVELRCDPISGHTALYWYRQSLGGQPEFLIYFQGTGAADDSGLPNDRFFAVRPEGSVSTLKIQRTERGDSAVYLCAS									
HUVB6.2/3	VV.GF..T.....S.RY..AKR.QD.A.....VS.F.Q.A.....T...NEAQL.K...S.....E.....QQE.....									
HUVB6.4	S.....VV.GF..T.....S.RY...KR.QD.A.....VS.....A.....T...NYEAGQ.K.....S.E.....I...T.....QR...M.R.AS									
HUVB6.5	-----ADT...N.RH.I.KR.QN.TF.....E.NR.....T...NEAQLEK.R.LS...S.E.K.L...E.....Q...M.....									
6.5b	E									
HUVB6.9N.RHNI.KR.QN.TF.....E.NR.....T...NEAQLEK.R.LS...S.E.K..E...E.....Q...M.....									
HUVB6.7F.V.F.....S.....D.....L.....NS.P.K....S...S.E.TG.....T....QQE.....									
6.7b	B									
HUVB6.8S.....M.....QEIS...HN.RH.I.KR.QN.TF.....E.NR.....NP.....T...NEAQLEK...LS..IS.E.K..F...E.....Q...M.....									
HUVB6w1S.RY...KR.QD.T.....S.VT....Q.A.....T...NYEAQPKD....S...S.E.....I...T.....QR...M.R...									

FIG. 2A

	1	10	20	30	40	50	60	70	80	90
HUVB13-1	NAGVTQTPKFQVLKTGQSM	TLQCAGDMNHEYMSWYRQDP	GMGLRIHYSVGAGITDQGE	VPNGYNVSRSTTFPLRLLSA	APQTSVYFCASS					
HUVB13-2R.....L.....Y.....E.T.AK.....D.....LKKQN.L.G.E.....				
HUVB13-3NS.Y.....Y.....ASE.T.K.....LNKRE.S.....E.....					
HUVB13-4HI.....G.L....P.....R.....A....K....D.....N.....E.....				
HUVB13-5	I..I..A.TS.I.AA.RR...	R.T...R.NA.Y....L.L.....	NT..T.GK....D..S...	AN.D....T.A..V.....						
HUVB13-6	-----T.....N..Y.....K..Y...P.....K.....EL.....	CL.....			
HUVB2.1a	MLLLLL	GLAGGLGAVVSGHP	SWICKSGTSVKIECRSLDFQ	ATTMFWRQFPKQSLMLMATS	NEGSKATYEQGVEKDKFL	INHASLTLSTLTVTSAHP	EDSSFYICS			
HUVB2.1bR.....K.....			
HUVB2.1cR.....			

4/5

HUVB5.1
MGSRLLCWVLLCLLGAGPVKAGVTQTPLYL IKTRGQQVTLS CSPISGHRSVSWYQQTPGGGLQFLFEYFSETQRNKGNFPGRFSGRQFSNSRSEMNVSTLELGDSALYLCASS

FIG.2B

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CDR3 REGIONS OF MONOCLONAL $V\beta 7$ CLONES FROM MS PATIENTS
GROWN IN IL-2 AND IL-4

Patient:		$V\beta$	N/D β /N	J β
82	7.1	CASS	QVPEGAL	GYTFSGSGTRLTVV-1.2
88	7.1	CASS	QD EG	YEQYFGPGTRLTVT-2.7
95	7.2	CASS	RHGRA	SYNEQFFGPGTRLTVL-2.1

CDR3 REGIONS OF OLIGOCLONAL $V\beta 7$ CLONES FROM MS PATIENTS

Patient:		$V\beta$	N/D β /N	J β	NOTES
81	7.2	CASS	QEGGVA	GELFFGEGSRLTVL-2.3	CD3+CD25+
	7.2	CASS	QEEHGG	TDTQYFGPGTRLTVL-2.3	"
	7.2	CASS	QDLTG	YNSPLHFGNGTRLTVT-1.6	"
	7.3	CASS	QDSVAY	SGNTIYFGECSWLTVV-1.3	"
88	7.2	CASS	QDRN	NEQFFGPGTRLTVL-2.1	CD4+CD25+
	7.1	CAS	QDRRVD	EAFFGQGTRLTVV-1.1	"
	7.2	CASS	QDGTGW	QPQHFGDGTRLSIL-1.5	"
	7.2	CAS	HGTSGIL	ETQYFGPGTRLLVL-2.5	"
	7.2	CASS	QGWG	TQYFGPGTRLLVL-2.5	"
66	7.1	CASS	QVAARPG	ELFFGEGSRLTVL-2.2	DIRECT SORT CD3+CD25+

FIG.3

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 94/04789

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 39/00, C 12 Q 1/68

According to International Patent Classification (IPC) or to both national classification and IPC 5

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K, C 12 Q, C 07 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A2, 92/12 996 (THE IMMUNE RESPONSE CORPORATION) 06 August 1992 (06.08.92), abstract; claims.	1-16
P, A	WO, A2, 93/12 814 (THE IMMUNE RESPONSE CORPORATION) 08 July 1993 (08.07.93), abstract; claims.	1-16
A	WO, A1, 91/17 268 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 14 November 1991 (14.11.91), abstract; claims 1, 3, 8, 9, 11.	1-16

☐ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

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Date of the actual completion of the international search

22 August 1994

Date of mailing of the international search report

- 9. 09. 94

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SCHNASS e.h.

ANHANG

ANNEX

ANNEXE

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

to the International Search
Report to the International Patent
Application No.

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 94/04789 SAE 90177

In diesem Anhang sind die Mitglieder
der Patentfamilien der in obenge-
nannten internationalen Recherchenbericht
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dans le rapport de recherche inter-
national visés ci-dessus. Les renseigne-
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In Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WD A2 9212996	06-08-92	AU A1 12716/92 CA AA 2101065 EP A1 568623 NO A0 932631 NO A 932631	27-08-92 23-07-92 10-11-93 21-07-93 21-09-93
WD A2 9312814	08-07-93	AU A1 34188/93 WD A3 9312814	28-07-93 30-09-93
WD A1 9117268	14-11-91	AU A1 78729/91 CA AA 2081672 EP A1 527199 EP A4 527199 JP T2 5506995	27-11-91 02-11-91 17-02-93 03-03-93 14-10-93